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Genetic Diversity in the Threatened Local Endemic Plant *Arisaema minus* (Araceae)

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The genetic diversity and genetic structure of the locally endemic *Arisaema minus* was examined using allozyme polymorphisms. *Arisaema minus* is restricted to Hyogo Prefecture in western Honshu, Japan. The Red List of Japan treats it as “vulnerable”. To determine the genetic diversity of the species, 274 individuals from six populations were sampled. Genetic diversity at 10 allozyme loci was relatively high at both the population and species level, and comparable to previously reported species of Japanese *Arisaema* species having wider distribution ranges. Although the western and eastern Hyogo populations are disjunct, very low genetic differentiation was observed among the six populations ($Gst = 0.06$). The genetic data suggest that the risk of rapid extinction of *A. minus* is quite low. The eastern Hyogo populations, however, have lower genetic diversity than those in western Hyogo and are threatened by habitat destruction caused by human activities, therefore placing them at greater risk of extinction. Periodic monitoring of both the demography and genetic variation in the eastern populations is needed.

Key words: *Arisaema minus*, genetic diversity, local endemic

Arisaema minus (Serizawa) J. Murata is a perennial, understory herb of secondary temperate forests *Quercus serrata* Murray, *Q. myrsinifolia* Blume, or *Cryptomeria japonica* (L.f.) D. Don plantations (Kobayashi 1992, 2000) and is endemic to Hyogo Prefecture in western Honshu, Japan. *Arisaema minus* belongs to the *A. undulatifolium* group, which is characterized by the spathe opening before extension of the leaves, a short rachis between the leaflets and the highest number of ovules per ovary within section *Pistillata* (formerly *Pedatisecta*) (Murata 1990, Kobayashi *et al.* 2003). Except for *A. nambae*, all member of the section have a chromosome number of $2n = 26$ (Watanabe *et al.* 1998). The *A. undulatifolium* and the *A. serratum* groups, characterized by high morphological diversity and the low genetic diversity, are considered to be closely related (Murata & Kawahara 1995).

The red list of Japan treats *Arisaema minus* as “vulnerable” (Environment Agency of Japan 2007). Populations of *A. minus* are disjunct in western (Sayo-cho, altitude 300-500 m) and eastern Hyogo (around Kobe city, altitude 100–400 m) (e.g., Kobayashi 2004). The eastern Hyogo populations of *A. minus* are threatened by habitat decline and population fragmentation through human activity. One population in particular was transplanted to prevent its loss due to highway construction (Marui *et al.* 2004). To conserve the endangered *A. minus*, a knowledge of its ecology and genetics is essential. Although details about habitat preference and reproduction have been reported (Kobayashi 1995, Marui *et al.* 2004, Yamazaki *et al.* 2004), the extent of genetic variation in *A. minus* is unknown. In particular, genetic information is essential for long-term conservation, because persistence of genetic diversity is necessary to allow species to adapt to changing environments (Hamrick & Godt 1995).

In this paper, we report on a genetic analysis of six populations using allozyme polymorphisms to obtain fundamental information on the extent of genetic variation in the endangered *A. minus*.

Materials and Methods

We sampled 274 individuals from six populations of *Arisaema minus* (Fig. 1, Table 1). Popula-

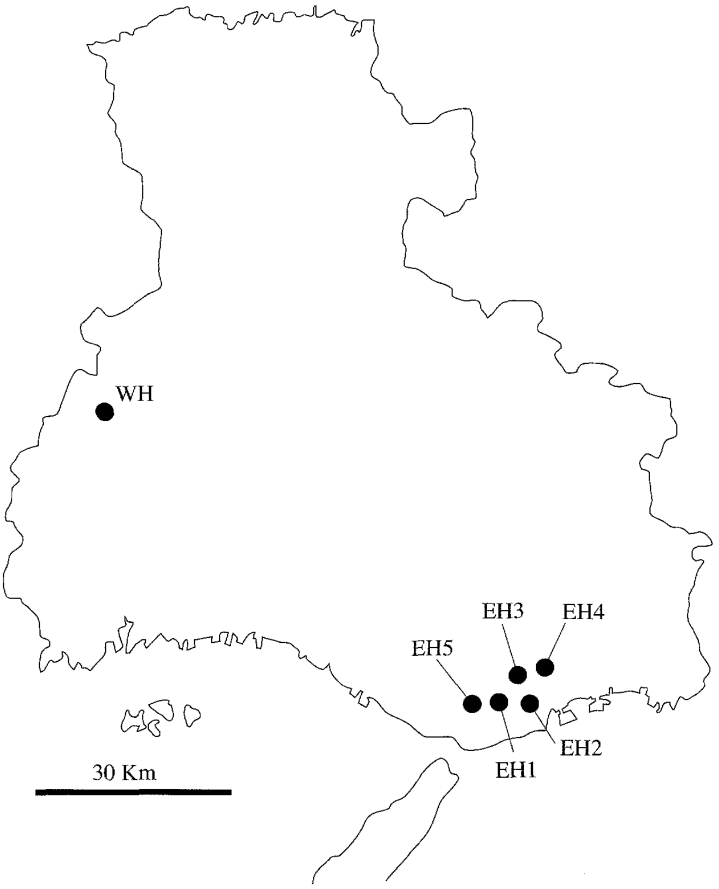

FIG. 1. Distribution of six populations *Arisaema minus* examined. Population codes and localities are summarized in Table1.

TABLE 1. Population Codes, localities, sample number (*N*), proportion of polymorphic loci (*P*), mean number of allele (*A*) and gene diversity within a population (*h*) at 10 loci examined in populations of *Arisaema minus*.

Population code	localities (altitude)	*N*	*P*	*A*	*h*
WH	Sayo-cho (300–500 m)	80	0.80	3.3	0.313
EH1	Suma-ku, Kobe (150–250 m)	40	0.60	3.0	0.280
EH2	Hyogo-ku, Kobe (250–350 m)	41	0.60	3.1	0.306
EH3	Kita-ku 1, Kobe (300–400 m)	38	0.50	2.3	0.254
EH4	Kita-ku 2, Kobe (300–400 m)	34	0.60	2.7	0.243
EH5	Nishi-ku, Kobe (100–200 m)	41	0.70	2.8	0.287
Mean			0.63	2.8	0.281
At the species level			0.80	4.0	0.374

tion codes and the number of individuals sampled are shown in Table 1. For each plant, 100 mg of fresh leaves were homogenized in 1000 μL of an extract buffer of 93 mmol/L Tris HCl (pH 7.5), 23.4% glycerol, 0.5% (v/v) Tween 80, 2.8 mmol/L dithiothreitol, and 0.5% 2-mercaptoethanol (Uchida *et al.* 1991). The homogenate was centrifuged at 20,000 × g at 4°C for 15 min. The resulting supernatant was used as a crude extract of the enzymes. Polyacrylamide vertical slab gel electrophoresis was carried out according to the procedures described by Davis (1964) and Ornstein (1964). Ten microliters of the crude enzyme extract were used for polyacrylamide gel electrophoresis. Eight enzyme systems were examined: aspartate amino-transferase (AAT, EC 2.6.1.1), alcohol dehydrogenase (ADH, EC 1.1.1.1), glutamate dehydrogenase (GDH, EC 1.4.1.2), leucine aminopeptidase (LAP, EC 3.4.11.1), phosphoglucoisomerase (PGI, EC 5.3.1.9), phosphoglucomutase (PGM, EC 5.4.2.2), shikimate dehydrogenase (SKDH, EC 1.1.1.25), and triose-phosphate isomerase (TPI, EC 5.3.1.1). Staining protocols followed those of Tsumura *et al.* (1990)

Based on the results of allozyme electrophoresis, the allele frequency in each population of *A. minus* was calculated for the loci encoding the eight enzyme systems. The following indices were used to quantify the amount of genetic diversity within each population examined: the proportion of polymorphic loci (P) at the 95% criterion, the number of alleles per locus (A), and the expected heterozygosity (h). Genetic diversity parameters (P, A, and h) were also calculated at the species level. As in Hamrick & Godt (1989), we treated the loci that were polymorphic in at least one population as polymorphic at the species level. Genetic diversity and genetic differentiation among the populations of *A. minus* were estimated by Nei's gene diversity statistics (Nei 1973).

Genetic identity (I) and standard genetic distance (D) (Nei 1972) were calculated for each pair-wise comparison of all populations examined.

Results and Discussion

A total of 10 putative loci was scored: *Adh*, *Aat-1*, *Aat-2*, *Gdh*, *Lap*, *Pgi*, *Pgm*, *Skdh*, *Tpi-1*, and *Tpi-2* for *A. minus* (Appendix 1). Among the 10 loci scored, two loci, *Gdh* and *Tpi-2*, were monomorphic in all populations. The other eight loci were polymorphic in at least one population. Table 1 summarizes the values of P, A, and h for each population and at the species level of *A. minus*. At the population level, the mean values of P, A, and h in *A. minus* were 0.63, 2.8, and 0.281, respectively. At the species level, the values of P, A, and h were 0.80, 4.0, and 0.374, respectively. Although we examined rather few loci, each genetic diversity parameter (P, A, h) of *A. minus* was much larger than in other endemic plant species ($P = 0.263$, $A = 1.39$, $h = 0.063$: Hamrick & Godt 1989). The genetic diversity parameters of *A. minus* are comparable to those reported for *A. serratum*, *A. ehimense*, and *A. tosaense* (Maki & Murata 2001), all of which have wider distribution ranges. Like other species of *Arisaema*, *A. minus* is sequentially dioecious (Marui *et al.* 2004), and an obligatory outcrosser, which may explain the high genetic diversity.

In the six populations we examined, all populations showed high genetic diversity, although some of the populations in eastern Hyogo were fixed at *Aat-1* (EH1 and EH3) and *Tpi-1* (EH1–3 and EH5). Two populations, WH and EH2, were in mountainous regions where the habitat has not been threatened by human activities. The populations in eastern Hyogo are scattered in an area approximately 10 × 5 km^2. Four populations, EH1 and EH 3–5, are located on hills originally covered with secondary forests of *Quercus serrata*. For the last several decades those populations have undergone fragmentation following the construction of roads and housing.

The total genetic diversity (H_T) and the coefficient of gene differentiation (G_{ST}) overall for all populations of *Arisaema minus* was 0.230 and 0.062, respectively. The latter value indicates that 94% of the total genetic variation was shared by all six populations of *A. minus*, and the remaining

6% was partitioned in each population. The values show that genetic differentiation among the six populations is very low.

The mean values of *I* and *D* in the six populations of *Arisaema minus* were 0.97 and 0.03, respectively (Table 2). These values are comparable to those for intraspecific populations of other species of seed plant (e.g., Gottlieb 1981, Crawford 1983).

The six populations of *Arisaema minus* maintain relatively high genetic variation despite their relatively narrow range of distribution, suggesting that the risk of rapid extinction is currently quite low. The eastern Hyogo populations, however, showed lower genetic diversity than those in western Hyogo, and they still face habitat loss through human activities. The five eastern populations of *A. minus* are approximately 70–80 km from the population in western Hyogo (WH). *Arisaema minus* is pollinated by fungus gnats of the families Mycetophilidae and Phoridae (Yamazaki *et al.* 2004). The seeds are thought to be dispersed by the Brown-eared Bulbul (*Hypsipetes amaurotis*) (T. Kobayashi *personal observation*). Bulbuls usually transport seeds within several hundred meters (Fukui 1995). Gene flow mediated by pollen or seed dispersal across the long distance between the eastern and western populations is highly unlikely. Our analysis suggests that the populations in eastern Hyogo are at a rather higher risk of extinction than the western populations. Further periodical monitoring of both the demography and genetic variation in the eastern populations is needed.

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TABLE 2. Genetic identities (upper triangle) and genetic distances (lower triangle) for six populations of *Arisaema minus*.

	WH	EH1	EH2	EH3	EH4	EH5
WH	—	0.958	0.951	0.985	0.975	0.985
EH1	0.042	—	0.956	0.984	0.972	0.956
EH2	0.050	0.045	—	0.954	0.980	0.951
EH3	0.015	0.016	0.047	—	0.980	0.982
EH4	0.026	0.029	0.020	0.021	—	0.968
EH5	0.015	0.046	0.050	0.018	0.033	—

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APPENDIX 1. Allele frequencies for 10 loci in the *Arisaema minus*.

		WH	EH1	EH2	EH3	EH4	EH5
Aat-1	a	0.050	0.000	0.049	0.000	0.071	0.000
	b	0.931	1.000	0.951	1.000	0.871	0.915
	c	0.000	0.000	0.000	0.000	0.057	0.049
	d	0.019	0.000	0.000	0.000	0.000	0.037
Aat-2	a	0.025	0.050	0.256	0.000	0.033	0.081
	b	0.000	0.013	0.013	0.000	0.000	0.081
	c	0.938	0.813	0.679	0.987	0.967	0.797
	d	0.031	0.075	0.026	0.000	0.000	0.014
	e	0.006	0.050	0.026	0.013	0.000	0.027
Adh	a	0.263	0.359	0.275	0.286	0.343	0.110
	b	0.000	0.051	0.013	0.000	0.000	0.000
	c	0.000	0.154	0.000	0.214	0.000	0.098
	d	0.600	0.397	0.688	0.500	0.657	0.793
	e	0.000	0.038	0.025	0.000	0.000	0.000
	f	0.138	0.000	0.000	0.000	0.000	0.000
Gdh	a	1.000	1.000	1.000	1.000	1.000	1.000
Lap	a	0.032	0.013	0.049	0.000	0.014	0.061
	b	0.551	0.718	0.902	0.649	0.914	0.488
	c	0.070	0.154	0.000	0.068	0.043	0.085
	d	0.291	0.115	0.037	0.270	0.014	0.305
	e	0.032	0.000	0.012	0.014	0.000	0.000
	f	0.025	0.000	0.000	0.000	0.014	0.061
Pgi	a	0.075	0.013	0.207	0.000	0.114	0.000
	b	0.125	0.088	0.049	0.026	0.014	0.000
	c	0.050	0.075	0.012	0.039	0.057	0.098
	d	0.681	0.700	0.561	0.724	0.743	0.732
	e	0.044	0.125	0.012	0.145	0.000	0.122
	f	0.025	0.000	0.159	0.066	0.071	0.049
Pgm	a	0.080	0.075	0.146	0.027	0.014	0.037
	b	0.529	0.800	0.366	0.676	0.557	0.585
	c	0.355	0.125	0.366	0.297	0.371	0.378
	d	0.036	0.000	0.122	0.000	0.057	0.000
Skdh	a	0.031	0.013	0.000	0.000	0.000	0.000
	b	0.744	0.413	0.415	0.622	0.571	0.720
	c	0.225	0.550	0.512	0.351	0.429	0.280
	d	0.000	0.025	0.073	0.027	0.000	0.000
Tpi-1	a	0.138	0.000	0.000	0.000	0.000	0.000
	b	0.000	0.000	0.000	0.000	0.029	0.000
	c	0.863	1.000	1.000	1.000	0.971	1.000
Tpi-2	a	1.000	1.000	1.000	1.000	1.000	1.000